THE DIFFERENTIATION OF FECAL STREPTOCOCCI BY THEIR FERMENTATIVE REACTIONS IN CARBOHYDRATE MEDIA.*†

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The use of fermentative reactions of the streptococci in carbohydrate media for tracing out the systematic relationships within this group of organisms was first suggested by Gordon[1] in 1904. His suggestions were further worked out and elaborated by Houston[2] in the following year and by Andrewes and Horder[3] in 1906. The two last-named observers made a careful study of the records of the cultures which had been isolated by Gordon and Houston, and of a number of strains isolated by themselves from a large variety of sources. From the results of their observations they divided the group streptococci into seven sub-groups or species which they considered to be the main types or type centers about which the more variable forms were clustered. The first of these types fermented saccharose, salicin, and coniferin, and is referred to by them as *Str. equinus*. The second type, for which they proposed the name, *Str. mitis*, fermented saccharose, lactose, and salicin. The third type, *Str. pyogenes*, also fermented saccharose, lactose, and salicin but exhibited some minor variations of morphology and pathogenic properties. The fourth type, *Str. salivarius*, fermented saccharose, lactose, and raffinose. The fifth type, *Str. anginosus*, fermented saccharose and lactose. The sixth type, *Str. fecalis*, fermented saccharose, lactose, salicin, coniferin, and mannite. The seventh type, which corresponded to the pneumococcus, fermented saccharose, lactose, raffinose, and inulin. Andrewes and Horder also pointed out the scarcity of lactose fermenting strep-

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[1] *Rpt. of Med. Off. to Local Gov't Bd. Great Britain*, 1902–3, 32, p. 421; *ibid.*, 1903–4, 33, p. 38.

[2] *Ibid.*, 1904–5, 34, p. 326; *Rpt. to London County Council*, July 11, 1905.

[3] *Lancet*, 1906, 171, p. 708.

tococci in horse dung and street air. They found, however, that most of the strains isolated from human feces acted on this sugar. In the same year Houston showed that lactose fermenting strains were very numerous in cow dung but that mannite was not fermented by bovine types while 24 per cent of the human strains attacked this carbohydrate. Raffinose was fermented by the majority of bovine streptococci while but 32 per cent of the human strains fermented this sugar.

In 1910 Winslow and Palmer[1] suggested that these fermentative reactions of the fecal streptococci on various carbohydrates might be used as a means for differentiating between cultures derived from human and animal excreta. The importance to water analysts of a test of this nature can scarcely be overestimated since by this means it would be possible to differentiate between pollution of human and animal origin in water supplies. Winslow and Palmer carefully reviewed the work of the English observers and presented a study of 302 cultures of streptococci isolated by them from human, equine, and bovine feces. These cultures were grown in broth containing dextrose, lactose, saccharose, mannite, and raffinose, and the acidity produced by the fermentation of these different carbohydrates determined by titration. The data accumulated in these experiments were interpreted by the statistical or biometrical method which has been so ably applied to the systematic study of the family *Coccaceae* by the Winslows. The basis of this system rests upon the "study of the frequency with which certain characters or combinations of characters occur. The type centers 'or species' are defined by the occurrence of a large number of individuals with a given characteristic. These common types among such variable organisms as the bacteria may properly be considered as representing species about which the rarer varieties are grouped."

These observers conclude that the "presence of streptococci forming over 3.5 per cent of acid in dextrose broth would seem in general to be characteristic of human stools. Second, that raffinose fermenting forms appear to be more abundant in bovine than in human feces. Third, and of most importance, that mannite

[1] *Jour. Infect. Dis.*, 1910, 7, p. 1.

fermenting streptococci, which make up about one quarter of the human streptococci, are very rare in the feces of the cow and horse."

The report upon 100 additional strains from human feces and 71 from water was published by Houston[1] in 1910. In the same year Gordon[2] also reported the results of his investigation upon streptococci isolated from diseased throats. In 1912 there appeared several papers dealing with the systematic relationships of these organisms as differentiated by their fermentative powers in various carbohydrate media. Broadhurst[3] reported upon 100 strains isolated from milk and Hilliard[4] upon 65 strains from normal and diseased throats.

These papers and the earlier literature bearing upon the subject have been carefully examined and reviewed by Winslow[5] in a paper published the same year. This observer compared the relative merits of the methods used by the English and American workers, and, after an exceedingly careful analysis of the data published, has endeavored to formulate a rational and clear interpretation of the, up to that time, somewhat confused mass of facts. In general the conclusions of the American and English workers are in close agreement. Winslow has pointed out, however, that, on the whole, the work of the English observers has been somewhat less accurate than that of the Americans on account of the cruder methods of technic used. The English used litmus as an indicator of acid production. They added a few drops of litmus solution to their nutrient carbohydrate broth and determined the positive or negative fermentation simply by the change in color of the litmus media. Winslow and the other American observers have emphasized the importance of a quantitative acid determination and in their work carefully titrated their cultures against an N/20 sodium hydrate solution using phenolphthalein as an indicator. Winslow is inclined to attribute any discrepancy between the results of the English and American observers to the different methods of technic employed. For a detailed review of the entire literature on the subject the reader is referred to this excellent paper by Winslow.

[1] *Fifth Rpt. on Research*, Metropolitan Water Bd., London, 1910.

[2] *Rpt. Med. Off. to Local Gov't Bd., Great Britain*, 1910–11, 40, p. 302.

[3] *Jour. Infect. Dis.*, 1912, 10, p. 272.

[4] *Amer. Jour. Dis. of Children*, 1912, 3, p. 287.

[5] *Jour. Infect. Dis.*, 1912, 10, p. 258.

TABLE 1.

SHOWING THE ACIDITIES PRODUCED BY STREPTOCOCCI ISOLATED FROM HUMAN FECES IN DEXTROSE, LACTOSE, SACCHAROSE, MANNITE, AND RAFFINOSE BROTH.

Culture	Dextrose	Lactose	Saccharose	Mannite	Raffinose
1	3.4	2.3	2.1	1.3	—.5
2	3.7	2.3	1.9	1.4	—.5
3	3.6	2.5	1.4	1.6	—.5
4	3.8	2.3	2.3	1.4	—.5
5	3.9	2.4	2.7	1.5	—.5
6	3.9	2.2	1.8	1.6	—.5
7	3.8	2.5	2.1	1.6	—.5
8	3.8	2.6	2.2	1.5	—.5
9	2.9	1.5	—.5	—.5	—.5
10	2.6	0.1	—.3	—.5	—.5
11	2.3	1.1	—.4	—.5	—.5
12	5.1	2.5	—.5	—.4	—.5
13	3.0	2.3	2.4	—.5	—.2
14	2.9	2.3	2.5	—.5	—.5
15	3.5	—.2	—.5	2.4	—.5
16	0.5	—.2	—.5	—.5	—.5
17	0.2	0.1	—.5	—.5	—.5
18	1.9	—.1	—.5	—.5	—.5
19	2.6	2.2	2.6	—.5	—.5
20	3.4	2.1	—.5	—.5	—.5
21	3.4	2.4	—.5	1.4	—.1
22	3.1	2.5	2.0	1.4	—.2
23	3.3	2.3	1.8	1.4	0
24	3.2	2.4	1.9	1.2	—.2
25	3.4	2.2	1.7	1.4	0.3
26	3.2	1.4	1.8	1.4	0.3
27	3.1	2.3	2.2	1.5	—.2
28	3.7	2.1	2.2	1.5	—.5
29	3.6	2.4	2.1	1.6	0
30	3.6	2.5	2.3	1.6	0
31	3.3	2.4	2.1	1.5	.1
32	3.2	2.3	1.8	1.4	—.5
33	3.6	2.4	2.1	1.6	—.1
34	3.6	2.5	2.2	1.5	0.1
35	3.5	2.4	1.7	1.5	0.5
36	3.4	2.2	2.3	1.4	0.2
37	3.3	2.2	2.0	1.5	0
38	3.2	2.4	2.0	1.5	—.4
39	3.4	2.4	1.9	1.4	0.5
40	3.4	2.3	1.3	1.5	—.2
41	2.5	1.8	2.1	1.4	—.5
42	2.6	1.9	1.6	0.9	—.2
43	2.2	1.8	1.4	0.9	—.5
44	2.6	1.8	1.4	1.1	—.5
45	2.3	1.8	1.6	1.2	0.2
46	2.6	1.9	1.4	1.2	0.2
47	3.0	1.9	1.3	1.1	0.2
48	2.9	1.7	1.4	1.0	0.3
49	2.2	1.7	1.8	1.7	—.5
50	3.6	2.6	2.2	1.6	0.2
51	3.6	1.8	2.0	1.6	0.1
52	3.5	2.7	2.1	1.6	0.3
53	3.6	2.8	2.2	1.6	0.2
54	3.4	2.7	1.6	1.6	—.3
55	3.4	2.6	2.1	1.6	0.8
56	3.3	2.3	1.8	1.5	0.5
57	3.3	2.7	1.7	1.3	—.1
58	2.4	2.5	1.8	1.2	—.4
59	3.2	2.4	1.7	1.3	—.4
60	3.3	2.5	1.9	1.5	—.2
61	2.7	1.6	1.4	1.1	—.3
62	2.5	1.4	1.3	1.1	0.4
63	2.6	1.5	1.4	1.0	0.3
64	2.5	1.8	1.5	1.2	0.4
65	2.7	1.5	1.6	1.2	0.2
66	1.8	1.5	1.0	1.1	0.4
67	3.5	2.0	2.1	1.4	0.7
68	3.5	1.6	1.7	1.3	0.2
69	3.8	1.7	1.6	0.9	0.4
70	3.2	1.8	2.1	1.5	0.7

TABLE 1—*Continued.*

Culture	Dextrose	Lactose	Saccharose	Mannite	Raffinose
71	3.5	1.8	1.8	1.3	0.6
72	3.6	1.8	1.7	1.3	0.3
73	3.8	1.8	1.6	1.3	0.5
74	3.5	1.9	1.7	1.3	0.3
75	3.7	1.8	1.6	1.1	0.5
76	3.6	1.8	1.7	1.1	0.4
77	3.7	1.8	1.5	1.3	0.5
78	4.0	1.9	1.5	1.3	0.4
79	3.8	1.8	1.7	1.3	0.6
80	3.7	1.8	1.8	1.3	0.4
81	3.9	1.7	1.7	1.2	0.4
82	3.4	1.8	1.8	1.3	0.5
83	3.8	1.9	2.0	1.4	0.2
84	3.6	1.8	1.5	1.4	0.5
85	3.6	2.2	1.8	1.2	0.5
86	3.8	1.8	1.8	1.3	0.7
87	3.6	2.1	1.8	1.1	0.7
88	3.5	1.8	1.6	1.2	0.6
89	3.6	2.0	1.9	1.2	0.6
90	3.7	1.7	1.7	1.3	0.1
91	3.6	1.8	1.7	1.3	0.9
92	3.7	1.8	1.9	1.1	0.5
93	3.5	1.8	2.0	1.5	0.9
94	3.7	1.9	1.6	1.4	0.7
95	3.9	2.2	1.7	1.4	0.5
96	3.6	1.8	1.3	1.5	0.5
97	3.5	1.9	2.3	1.5	0.5
98	3.4	2.0	1.7	1.5	0.5
99	3.6	2.2	1.9	1.5	0.6
100	3.8	2.2	1.5	1.3	0.6
101	3.2	3.2	1.6	1.3	0.6
102	3.9	2.6	1.8	1.4	0.6
103	3.0	1.9	1.8	1.2	0.2
104	3.7	2.1	1.5	1.5	−.5
105	3.5	2.2	1.9	1.2	0.6
106	4.7	2.1	1.6	1.5	0.4
107	2.9	2.2	1.7	1.4	0.5
108	3.2	1.8	1.8	1.1	0.2
109	3.8	2.1	1.7	1.3	1.4
110	3.9	2.4	2.1	1.8	0.3
111	3.5	1.5	1.5	1.5	0.6
112	3.0	2.1	1.7	2.1	0.5
113	3.7	2.9	1.7	1.3	0.4
114	3.0	2.1	0.8	1.3	0.4
115	2.9	2.8	1.7	1.4	0.5
116	2.7	2.0	1.9	1.1	0.3
117	3.5	2.4	1.6	1.1	0.4
118	3.4	2.4	1.5	1.4	0.6
119	3.7	2.7	2.4	1.7	0.6
120	3.8	1.7	1.7	1.3	1.0
121	4.0	2.2	1.7	1.5	0.5
122	3.9	2.0	5.3	1.1	0.4
123	1.8	0	0	0	0

Since the appearance of Winslow's paper two others have been published in this country, one by Bergey,[1] who regards the fermentative action of the streptococci on carbohydrates as too variable a quantity to be of value in the differentiation of these forms. He has reported entirely negative results. A second paper by Hilliard,[2] published in 1913, gives the results of the examination of 240 strains of streptococci isolated from normal and diseased

[1] *Jour. Med. Research*, 1912, 27, p. 67.

[2] *Jour. Infect. Dis.*, 1913, 12, p. 144.

TABLE 2.

SHOWING THE ACIDITY PRODUCED BY STREPTOCOCCI ISOLATED FROM HORSE DUNG IN DEXTROSE, LACTOSE, SACCHAROSE, MANNITE, RAFFINOSE, INULIN, AND SALICIN BROTH.

Culture	Dextrose	Lactose	Saccharose	Mannite	Raffinose	Inulin	Salicin
1	2.3	2.7	3.0	0.3	0.4	0.2	2.1
2	4.0	2.1	1.9	1.4	0.7	0.3	2.7
3	3.2	3.0	1.0	2.1	0.6	0.4	2.6
4	4.2	3.0	2.2	2.2	0.6	0.4	2.5
5	3.6	3.0	3.2	1.6	0.8	0.2	2.2
6	1.7	2.3	1.6	0.1	0.5	2.1	0.6
7	3.9	1.6	3.4	0.3	1.4	0.3	3.4
8	4.1	0.9	3.3	0.3	0.3	0.1	2.8
9	3.6	2.5	2.9	1.1	0.3	0.1	2.8
10	3.6	2.3	3.5	0.1	0.3	0	2.0
11	4.6	1.9	3.1	−.5	0.8	−.3	2.6
12	1.6	0.4	1.0	0.2	0.4	2.1	0.5
13	1.4	0.4	1.7	0.4	0.7	2.0	0.7
14	1.5	0.8	1.8	0.3	0.5	−.5	1.9
15	1.7	0.7	1.2	0.4	0.1	0.1	1.2
16	1.3	0.5	0.9	0.5	0.6	0.1	1.2
17	1.5	0.7	1.3	0.4	0.6	1.6	1.2
18	1.5	1.3	2.8	0.3	0.3	2.9	1.7
19	1.4	0.8	1.2	0.4	1.2	0.9	1.5
20	1.3	0.4	1.5	0.1	1.0	4.0	1.4
21	1.7	1.9	0.1	0.5	0.7	0.1	0.5
22	1.5	0.6	1.2	0.4	1.2	0.3	1.1
23	1.3	0.6	0.8	0.4	0.9	0.3	1.2
24	1.7	0.4	0.9	0.5	0.4	0.3	1.3
25	2.7	0.5	2.3	0.1	0.5	0.2	2.2
26	1.1	0.3	1.2	0.3	0.5	0	0.1
27	2.7	1.3	3.1	1.3	2.5	3.7	1.6
28	1.2	0.3	0.9	0.2	0.3	0.3	0.8
29	2.5	2.0	2.7	0.4	2.7	3.0	2.5
30	2.3	0.9	2.1	0.2	2.4	3.1	2.8
31	1.3	0.2	0.5	0.1	0.2	0.1	1.1
32	1.9	1.4	2.1	0.4	1.7	3.2	2.2
33	1.4	1.3	0.8	0.2	−.7	1.0	1.1
34	2.2	3.3	2.7	1.0	2.2	2.9	2.6
35	0.6	1.2	1.0	0.5	0.7	0.5	−.1
36	1.5	0.3	1.0	0.5	0.5	0.1	0.9
37	2.2	1.5	1.0	0	0.9	−.1	1.3
38	2.6	0.5	0.6	0	0.9	1.6	1.6
39	3.4	0.2	0.6	0.3	0.6	1.1	0.6
40	1.5	0.2	0.6	0.5	0.9	2.2	0.6
41	1.4	0.4	1.2	0.3	0.7	1.3	1.2
42	1.2	1.2	1.2	0	0.8	0.0	1.3
43	1.5	0.4	1.4	0.0	0.5	0.5	1.4
44	5.3	−.4	2.3	0.0	2.4	0.6	2.4
45	1.6	0.3	1.5	0.3	0.7	2.0	1.4
46	1.6	0.5	1.4	−.3	1.0	0.2	1.9
47	1.6	0.2	1.7	0.0	0.6	2.0	1.3
48	1.4	0.5	1.8	0.3	0.4	1.5	2.0
49	1.3	1.8	1.2	0.1	1.6	1.5	1.7
50	1.9	0.5	1.1	0.0	1.9	0.3	1.0
51	1.5	0.3	1.4	0.0	0.7	1.3	1.4
52	2.0	1.0	1.3	0.0	0.8	0.9	1.3
53	1.5	0.5	1.3	0.0	1.1	1.8	1.4
54	1.3	1.8	1.4	0.3	1.3	1.8	1.2
55	1.6	0.2	1.5	0.0	0.8	1.4	0.9
56	1.8	0.8	1.3	0.0	0.4	1.0	1.1
57	1.3	1.9	2.0	−.2	1.5	1.1	1.9
58	1.2	0.2	1.2	−.4	1.0	1.0	1.3
59	1.5	0.6	1.2	0.7	1.0	0.0	1.4
60	1.5	0.7	1.4	−.4	0.5	2.5	1.4
61	1.5	0.5	1.6	0.0	0.6	1.2	1.2
62	1.7	1.3	1.2	0.0	0.9	1.2	1.3
63	1.2	0.5	0.9	0.0	0.5	0.0	1.0
64	1.3	0.7	1.5	0.0	0.5	1.3	1.1
65	1.8	0.2	1.1	0.0	0.9	0.7	1.4
66	1.5	0.7	1.3	0.0	0.5	0.7	1.4
67	1.7	0.5	1.3	0.0	1.7	0.3	0.3
68	1.5	0.7	1.7	0.0	1.3	0.8	1.7
69	1.5	0.5	1.2	0.0	0.6	1.1	1.2
70	1.3	0.5	1.2	−.2	1.0	1.2	1.5

TABLE 2—*Continued.*

Culture	Dextrose	Lactose	Saccharose	Mannite	Raffinose	Inulin	Salicin
71.....	1.5	0.6	1.0	0.0	0.7	0.8	1.3
72.....	1.4	1.6	1.1	0.0	1.0	0.1	0.8
73.....	1.4	0.5	1.0	0.0	0.5	0.7	1.0
74.....	1.9	0.5	1.2	0.0	0.6	0.1	1.3
75.....	1.3	0.5	0.9	0.0	0.3	0.0	1.2
76.....	1.5	0.5	0.9	0.0	0.5	1.3	0.5
77.....	1.4	1.7	1.4	−.3	0.6	0.2	1.2
78.....	3.8	1.9	2.4	1.6	0.5	0.2	2.0
79.....	1.5	0.5	1.5	0.0	1.2	0.2	0.7
80.....	1.4	1.5	1.2	0.0	0.9	1.4	0.9
81.....	3.8	2.0	2.6	1.5	0.5	0.3	1.8
82.....	1.2	0.5	0.9	0.0	1.1	1.0	0.5
83.....	1.5	0.1	1.1	0.0	1.1	1.2	0.9
84.....	1.5	0.3	1.5	0.7	1.0	0.2	1.2
85.....	2.1	0.7	2.0	0.4	1.3	1.7	1.4
86.....	1.6	0.5	1.5	0.1	0.5	1.4	1.8
87.....	1.8	0.4	1.0	0.9	0.7	2.5	1.4
88.....	1.6	0.6	1.4	0.9	0.5	2.6	1.3
89.....	1.9	0.6	0.8	0.2	0.6	0.6	2.3
90.....	1.6	0.3	1.6	−.2	0.7	1.4	0.9
91.....	1.6	2.6	2.6	1.0	0.3	3.8	1.6
92.....	1.6	0.5	1.1	0.0	0.7	0.6	1.1
93.....	1.8	0.5	1.0	0.1	0.4	1.0	0.6
94.....	1.7	0.3	1.5	0.5	0.5	0.8	1.0
95.....	1.9	1.7	1.4	−.1	1.1	1.1	1.2
96.....	1.7	0.5	0.7	−.1	0.8	0.5	0.5
97.....	1.5	0.3	0.9	0.1	0.3	1.7	0.5
98.....	1.8	0.3	1.7	0.0	0.4	1.7	1.7
99.....	1.9	1.7	1.3	1.2	0.6	0.8	0.8
100.....	1.1	1.7	0.5	0.2	0.9	1.1	1.0
101.....	1.7	1.3	1.6	1.0	0.9	2.3	1.4
102.....	−.3	0.3	1.5	−.1	0.2	0.7	0.9
103.....	1.4	1.7	0.5	0.0	1.1	0.8	0.7
104.....	2.3	1.9	1.6	1.3	1.3	1.5	2.0
105.....	3.1	0.7	2.3	1.2	2.6	3.2	−.3
106.....	1.4	0.3	1.4	−.2	0.4	0.5	1.3
107.....	0.6	0.4	0.6	0.8	0.4	0.6	0.5
108.....	1.5	0.5	1.6	0.0	0.5	2.1	0.9
109.....	1.5	0.2	1.4	0.0	0.0	0.5	1.7
110.....	0.9	1.1	1.0	−.1	0.6	0.7	1.0
111.....	1.5	2.3	1.8	−.2	0.7	1.3	0.9
112.....	1.7	0.6	1.7	0.0	0.5	0.5	1.4
113.....	1.0	0.6	0.5	0.5	0.1	0.5	0.6
114.....	1.2	0.5	0.8	0.0	0.4	1.6	0.5
115.....	1.6	0.4	1.7	0.1	0.5	1.9	1.0
116.....	1.7	0.7	1.7	−.4	1.2	0.6	1.7
117.....	1.5	0.6	0.9	−.1	0.4	0.5	1.2
118.....	1.6	0.5	1.2	−.2	0.4	1.1	1.1
119.....	1.4	0.5	1.3	−.1	0.7	0.9	0.3
120.....	0.8	0.4	0.9	0.7	0.7	0.4	0.8
121.....	1.0	0.6	1.2	0.2	0.2	0.3	0.7
122.....	1.2	0.5	0.6	0.1	0.2	0.6	0.5
123.....	1.4	0.9	1.3	−.3	0.3	0.7	0.5
124.....	1.7	0.5	1.6	−.1	0.4	1.4	0.8
125.....	1.4	0.4	1.1	0.1	0.8	0.6	1.0
126.....	0.8	0.7	0.7	−.1	0.5	0.7	0.5
127.....	0.2	0.7	0.4	0.1	0.1	0.8	0.8
128.....	0.9	0.6	0.5	0.4	0.4	0.5	0.0
129.....	1.6	0.9	1.6	0.4	0.8	1.3	0.0

throats and from milk. His results on the whole compare very favorably with those of Winslow and Broadhurst.

With the exception of the papers by Winslow and Palmer and by Houston in 1910 most of the research upon streptococci has been made primarily to determine the systematic relationships of these organisms rather than to formulate a practical method for the

differentiation of streptococci characteristic of human and animal excreta. In the majority of these investigations the cultures were isolated from milk, normal and diseased throats, and various streptococcic infections of tissues.

The studies upon streptococci presented in this paper were undertaken with the idea of following out Winslow's suggestion for using the fermentative action of streptococci in carbohydrate media as a means of differentiating between human and animal pollution in water supplies. In these experiments we have confined ourselves entirely to the study of fecal strains and have sought to approach the problem from the viewpoint of the sanitary water analyst rather than with the idea of adding to the knowledge concerning the systematic relationships of the group streptococci. In order to familiarize ourselves with Winslow's technic and at the same time to supply additional data for purposes of comparison we have tested the fermentative action of a number of cultures of fecal streptococci upon several carbohydrates before proceeding to the application of the test to the routine examination of water. This paper presents a preliminary report on the study of 350 strains of streptococci isolated from human, equine, and bovine feces. One hundred and twenty-three of these cultures were obtained from human feces; 129 from horse dung; and 98 from cow dung.

METHODS.

The methods used in these experiments are essentially those employed by Winslow and Broadhurst. The media used were prepared from beef extract by the usual method and the reaction adjusted to 0.5 per cent plus to the phenolphthalein scale. The cultures were isolated by plating direct on plain agar without previous enrichment in broth. After 24 hours' incubation at 37.5° C. characteristic colonies were transferred to tubes of maltose broth, and agar slope cultures made at the same time. If, after 24 hours' incubation, the agar slopes developed a scanty veil-like growth and the microscopic examination of the growths in the broth tubes revealed the presence of chains of spherical cells, the cultures were considered positive. Transfers were then made into broth containing one per cent of the carbohydrate used. The fermentative properties of the strains isolated from human feces were tested in dextrose, lactose, saccharose, raffinose, and mannite broth. Those isolated from the horse and cow were tested in broth containing the above carbohydrates and in inulin and salicin broth in addition. After three days' incubation at 37.5° C. the acidity produced through the decomposition of the various carbohydrates was determined by titration.[1] Five cubic centimeters of

[1] Our acknowledgments are due to Miss L. W. Fox and Mr. C. I. Nelson for assistance in some of the titrations for acidity.

TABLE 3.

SHOWING THE ACIDITY PRODUCED BY STREPTOCOCCI ISOLATED FROM COW DUNG IN DEXTROSE, LACTOSE, SACCHAROSE, MANNITE, RAFFINOSE, INULIN, AND SALICIN BROTH.

Culture	Dextrose	Lactose	Saccharose	Mannite	Raffinose	Inulin	Salicin
1	2.0	2.2	1.6	1.4	1.8	2.7	1.7
2	0.6	0.6	0.2	0.6	0.4	0.6	0.6
3	1.8	1.9	0.8	2.3	1.7	2.7	2.4
4	1.8	2.0	1.9	0.3	1.9	3.1	1.6
5	1.9	3.1	0.8	0.4	1.6	2.9	2.0
6	2.0	2.5	2.0	0.1	1.7	2.8	1.3
7	2.3	2.2	1.7	0.2	1.9	1.7	1.6
8	2.3	2.1	0.8	0.5	1.7	1.5	1.7
9	1.9	1.9	1.6	0.2	1.7	2.2	1.5
10	1.6	2.0	0.8	0.1	1.2	2.3	1.7
11	1.7	0.8	0.3	0.3	0.3	0.7	0.6
12	2.5	1.9	1.8	0.6	1.5	2.2	1.7
13	0.6	1.6	1.9	0.1	1.6	1.7	2.2
14	0.7	0.6	0.3	0.7	−.1	0.4	0.4
15	2.0	1.8	1.7	−.1	1.8	2.0	2.1
16	0.6	0.6	0.0	0.1	0.3	0.8	0.5
17	2.0	1.9	1.6	0.0	1.4	1.7	2.2
18	2.0	2.0	2.0	0.0	1.4	1.6	1.9
19	3.1	2.2	2.2	0.1	0.5	0.7	1.4
20	0.5	0.6	0.2	0.1	0.2	0.7	0.5
21	1.6	1.9	1.7	0.2	1.5	2.7	2.3
22	4.4	4.0	4.3	0.5	3.3	4.0	4.5
23	2.1	1.0	1.5	0.2	2.0	2.7	1.8
24	1.9	1.8	1.7	0.1	−.4	2.8	1.7
25	2.5	2.4	1.9	0.3	2.0	3.1	2.3
26	1.9	1.9	1.8	0.5	1.3	3.5	1.7
27	2.3	2.5	1.7	0.2	1.4	3.3	2.1
28	2.9	3.0	1.6	0.1	1.7	2.8	1.7
29	2.8	2.3	1.5	0.3	1.4	2.0	1.5
30	2.2	2.2	1.8	0.5	1.5	2.8	1.5
31	0.9	0.8	0.5	0.4	1.0	0.2	0.7
32	2.9	2.1	2.7	0.2	3.0	2.6	3.0
33	2.2	2.1	2.5	0.2	2.3	3.4	2.6
34	4.3	3.0	2.6	0.3	3.3	0.4	2.0
35	2.6	2.2	2.6	0.1	2.6	3.4	2.6
36	2.1	2.1	2.6	0.3	3.1	2.7	2.5
37	1.9	2.2	2.5	0.3	3.2	3.7	2.4
38	2.2	2.5	3.0	0.5	3.3	2.5	2.9
39	2.0	2.0	2.6	0.4	4.3	3.7	2.7
40	1.6	1.8	2.5	1.3	0.6	0.9	1.5
41	2.9	2.4	2.8	0.2	3.2	3.8	2.8
42	2.8	3.0	2.8	0.2	3.7	2.8	3.0
43	1.7	0.7	1.3	1.0	0.9	1.0	1.1
44	2.8	1.6	2.8	0.6	3.2	3.7	2.8
45	1.3	1.2	1.1	0.9	1.1	1.1	1.3
46	1.1	0.8	1.1	1.3	1.2	1.3	1.2
47	3.2	2.1	3.6	1.5	0.4	1.7	2.0
48	0.4	0.4	0.1	0.2	0.1	0.3	0.2
49	2.6	2.4	2.3	0.8	2.4	2.9	2.9
50	2.4	2.8	2.4	1.0	3.0	3.1	2.6
51	0.7	0.7	0.4	0.6	0.4	0.2	0.2
52	3.0	2.6	2.4	0.6	2.8	2.7	3.2
53	2.4	2.0	2.3	0.7	2.6	3.5	2.0
54	0.4	0.4	0.1	0.1	0.0	0.7	0.3
55	2.8	2.4	2.3	0.5	2.6	3.4	2.7
56	2.2	2.5	2.4	2.5	2.3	2.6	2.4
57	2.2	1.5	2.3	0.5	2.3	3.5	2.5
58	2.7	2.5	2.6	0.6	2.5	2.9	2.3
59	2.8	2.5	3.0	0.7	2.7	3.6	0.9
60	2.5	2.5	2.3	0.7	1.9	2.6	2.4
61	2.9	2.4	2.6	0.8	3.4	3.1	2.2
62	2.4	2.1	2.5	0.8	3.5	3.2	2.8
63	2.5	2.6	2.0	0.6	2.6	2.6	2.6
64	2.1	2.7	2.6	0.7	2.1	3.1	3.0
65	2.9	2.2	2.7	0.7	2.3	3.0	2.0
66	2.4	2.4	2.6	0.7	2.0	2.7	2.2
67	2.4	2.4	2.7	1.0	2.7	3.8	3.0
68	2.0	1.7	1.9	0.2	2.0	2.3	1.6
69	1.5	1.8	2.1	0.4	1.9	2.7	1.6
70	1.4	1.5	2.1	0.2	2.5	1.6	2.0

TABLE 3—*Continued.*

Culture	Dextrose	Lactose	Saccharose	Mannite	Raffinose	Inulin	Salicin
71.....	2.3	2.2	2.5	0.5	2.9	3.5	1.7
72.....	1.6	1.8	2.4	0.5	2.9	3.0	1.7
73.....	1.8	2.0	2.1	0.0	2.4	3.0	1.9
74.....	0.8	1.8	2.3	0.3	2.8	3.2	1.7
75.....	1.5	1.8	1.8	0.2	1.9	3.0	1.7
76.....	3.2	1.5	0.6	1.6	0.3	1.5	1.1
77.....	1.7	1.8	2.2	0.0	2.5	2.9	1.8
78.....	1.0	1.2	1.5	0.6	0.4	0.5	0.6
79.....	1.6	1.7	2.2	0.0	2.5	3.0	1.8
80.....	1.6	0.9	2.2	0.0	2.2	2.7	0.8
81.....	0.5	1.4	1.0	0.7	0.7	0.5	0.6
82.....	1.7	1.6	1.8	0.0	2.6	3.2	1.8
83.....	1.5	1.7	2.3	0.1	2.3	3.3	1.6
84.....	1.5	1.7	2.3	0.1	2.4	3.4	1.6
85.....	1.8	1.8	1.5	0.3	2.8	2.9	1.3
86.....	1.9	1.9	2.1	0.3	2.7	3.8	1.8
87.....	1.7	1.7	1.7	0.1	2.0	3.1	0.7
88.....	0.5	1.3	2.5	0.2	2.2	2.7	0.6
89.....	0.4	0.2	0.2	0.1	1.1	0.4	0.2
90.....	1.8	1.2	2.0	0.1	1.9	2.7	1.7
91.....	1.7	1.3	1.9	0.4	1.9	3.2	0.3
92.....	1.9	—.1	2.4	0.5	2.0	2.9	1.7
93.....	0.4	0.1	0.0	0.0	0.2	0.5	0.2
94.....	2.9	0.0	1.0	0.9	0.3	0.3	0.3
95.....	1.9	2.1	1.1	0.5	1.0	2.5	1.7
96.....	2.5	1.7	3.0	0.2	2.4	2.4	1.0
97.....	0.9	0.8	1.1	0.7	0.5	1.0	1.7

the broth culture were measured by means of a graduated pipette and added to 45 c.c. of distilled water in a 250 c.c. Phillips beaker. The acidity was then determined by titrating in the cold against an N/20 sodium hydrate solution using phenolphthalein as an indicator. During the process of titration the flasks were placed on white porcelain slabs and the faintest indication of a pink color in the solution was taken as the end point. Uninoculated tubes of broth were incubated and titrated with each series of inoculations made in order to keep a check upon the reaction of media used. The milk and neutral red tests recommended by the English observers were not employed in this work as it seemed to the writers that the information gained by the study of the coagulation of milk and the reduction of neutral red does not furnish important additional data for the differentiation of the various types of streptococci studied in these experiments.

RESULTS.

The results of the titrations to determine the acidities produced by streptococci isolated from human, equine, and bovine excreta are given in detail in Tables 1, 2, and 3. These tables give the actual acidity produced by the cultures after deducting the initial acidity of the medium in which they were grown. From the study of these figures it will be observed that the amount of acid produced by streptococci isolated from the same species does not vary greatly in a given carbohydrate. For example 91 out of 123 strains isolated from human feces produced between three and four per

cent of acid in dextrose broth. One hundred and two out of 129 strains from horse dung produced between 0.5 per cent and 2.0 per cent of acid in saccharose broth. And 90 out of 98 strains from cow dung produced less than one per cent of acid in a mannite broth. These relationships are brought out more clearly in Table 4 and in Charts 1, 2, 3, 4, and 5, which have been plotted from it. In this table the range of acidity produced by the fecal streptococci, extending from −0.5 to 5.5 per cent normal acid, is expressed by a series of groups, each group representing one-half per cent of acid.

TABLE 4.

STREPTOCOCCI GROUPED ACCORDING TO THE PERCENTAGE OF ACID PRODUCED IN DIFFERENT CARBOHYDRATE MEDIA.

	−.5 0	0.1 0.5	0.6 1.0	1.1 1.5	1.6 2.0	2.1 2.5	2.6 3.0	3.1 3.5	3.6 4.0	4.1 4.5	4.6 5.0	5.1 5.5
						(Human 123)						
Dextrose	0	2	0	0	2	7	13	34	40	0	1	1
Lactose	3	2	0	7	38	42	9	1	0	0	0	0
Saccharose	8	0	2	16	53	19	2	0	0	0	0	1
Mannite	9	0	4	72	13	2	0	0	0	0	0	0
Raffinose	36	45	14	1	0	0	0	0	0	0	0	0
						(Horse 129)						
Dextrose	1	1	6	46	29	5	2	2	5	2	1	1
Lactose	1	46	23	8	13	3	4	1	0	0	0	0
Saccharose	0	5	24	39	16	5	5	4	0	0	0	0
Mannite	55	38	7	5	2	2	0	0	0	0	0	0
Raffinose	1	39	40	13	4	4	2	0	0	0	0	0
Inulin	6	29	22	21	8	5	3	3	2	0	0	0
Salicin	3	12	23	36	14	6	5	1	0	0	0	0
						(Cow 98)						
Dextrose	0	6	9	6	32	22	15	3	0	2	0	0
Lactose	2	4	12	10	31	29	7	1	1	0	0	0
Saccharose	2	9	6	9	25	27	17	0	1	1	0	0
Mannite	9	57	25	4	1	2	0	0	0	0	0	0
Raffinose	3	13	5	12	22	16	15	9	1	1	0	0
Inulin	0	11	9	4	8	7	33	20	7	0	0	0
Salicin	0	11	10	11	34	15	15	1	0	1	0	0

The percentage of strains from each species is placed in the acidity-group which corresponds to the amount of acid produced in each of the carbohydrates tested. By this arrangement it is possible to see at a glance where the center of fermentative activity lies and to determine the frequency with which certain definite amounts of acid are produced in a series of strains from a given source in a given carbohydrate. It is evident that the cultures arrange themselves into two quite distinct groups of numerical frequency. One group was found in the neighborhood of no acid or of slight acid production, and the second at a point from one to three per cent higher

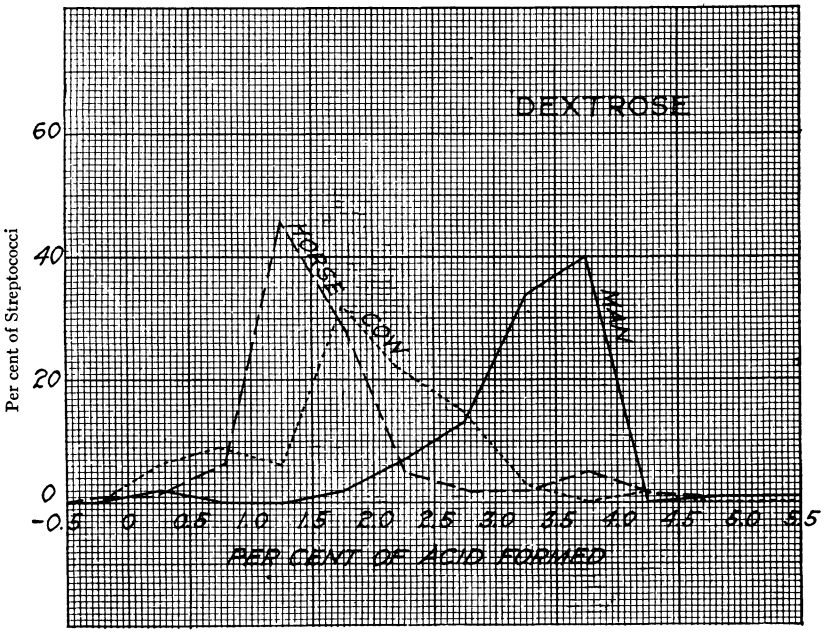

CHART 1.

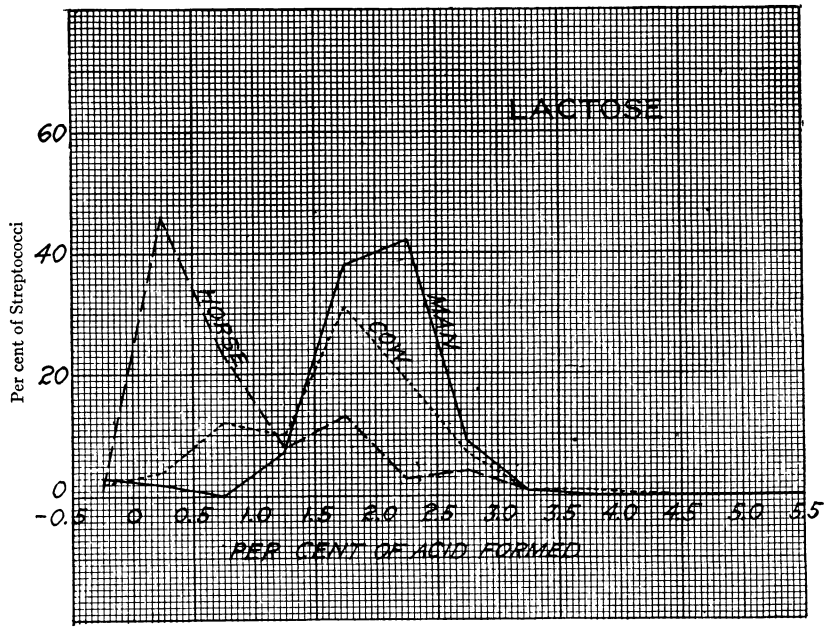

CHART 2.

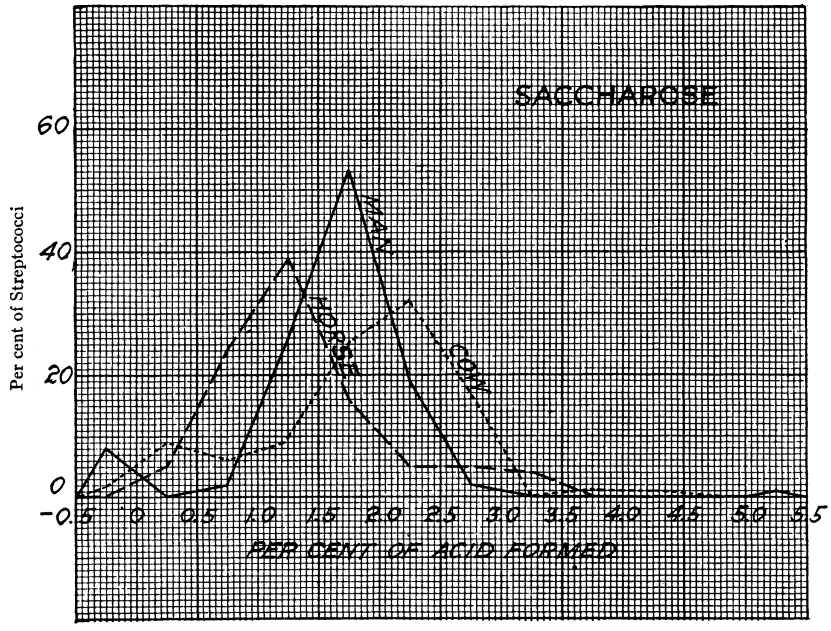

CHART 3.

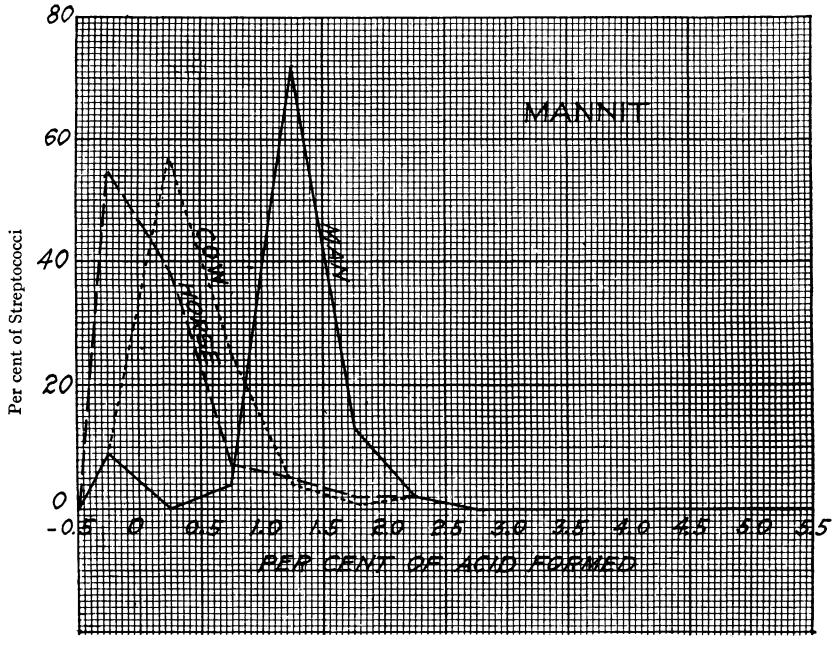

CHART 4.

in the scale of acidity. (See accompanying charts.) The same general condition was noted in each of the carbohydrates studied by us. This point was brought out by Winslow, Broadhurst, and Hilliard, who have shown that these two groups represent probably a non-fermenting and a fermenting type of organism. The division line between these two types in all cases falls between 0.5 and 2.0 per cent normal; thus, following the suggestion of Winslow, we have

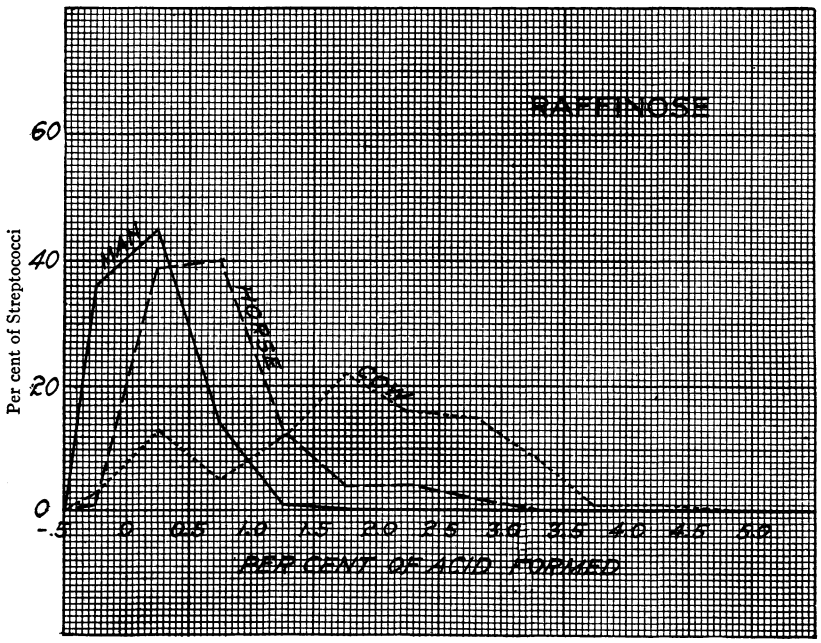

CHART 5.

considered as fermenting organisms only those which have produced over 1.2 per cent acidity. Those which produced 1.2 per cent or less we have classed among the non-fermenting types.

Our results show that dextrose is fermented by practically all strains of streptococci studied; 97 per cent of those isolated from human feces, 75 per cent of those from horse dung, and 81 per cent of those from cow dung acted upon this sugar. But the cultures from the human feces produced a considerably higher acidity than those from either the horse or cow. The great majority of human

strains produced between 3.5 per cent and 4 per cent of acid in this sugar; about 75 per cent of the streptococci from the horse produced between 1.0 per cent and 2.0 per cent; and 76 per cent of the cultures isolated from the cow produced between 1.5 per cent and 3.5 per cent of acid in dextrose.

Lactose is not attacked as readily as dextrose except possibly by streptococci from human and bovine feces. Ninety-four per cent of the human and 77 per cent of the bovine strains ferment lactose. There are comparatively few lactose fermenting streptococci in the excreta of the horse, only 24 per cent of the strains from this source being able to attack this sugar. The percentage of acid produced in lactose by streptococci from all these sources is not as high as in dextrose.

Saccharose is fermented by 90 per cent of the strains obtained from human feces, by 46 per cent of those from the horse, and by 75 per cent of those from the cow.

The streptococci that ferment mannite are found abundantly only in human feces. Sixty-five per cent of the cultures derived from this source fermented mannite more or less vigorously, while only 2 per cent of those from horse manure and 3 per cent of those from cow dung decomposed this substance. We have noted the presence of mannite fermenting streptococci in human feces in a consistently larger number of cases than is reported by Winslow.

No strains which fermented raffinose were isolated from human feces and only 12 per cent of those obtained from horse manure attacked this sugar. On the other hand, 73 per cent of the streptococci from cow dung fermented raffinose. In this respect also we obtained a larger number of raffinose fermenting streptococci from the cow than is noted by Winslow in his work. These results are not essentially different from those of either Winslow or Houston. Houston has omitted entirely the study of the action of streptococci on dextrose and Winslow has not tried out saccharose.

Table 5 shows the percentage of strains of streptococci fermenting different carbohydrates as found by Winslow, Houston, and Fuller and Armstrong.

Winslow has shown that the production of an acidity of 0.8 per cent in neutral litmus broth is sufficient to cause a distinct redding

of the litmus. Thus if the mere formation of acid is regarded as a positive test for fermentation the English observers have probably recorded as fermenting strains a number which would be considered non-fermenters if judged upon the basis of quantitative acid production. The somewhat higher figures recorded by Houston, Winslow attributes to the use of litmus as an indicator of the fermentative powers of the organisms tested.

TABLE 5.

	HUMAN			HORSE			COW		
CARBOHYDRATE	Winslow	Houston	Fuller and Armstrong	Winslow	Houston	Fuller and Armstrong	Winslow	Houston	Fuller and Armstrong
Dextrose	89	.....	95	65	.....	75	84	.....	81
Lactose	62	100	94	8	0	24	52	100	77
Saccharose	.....	85	90	.....	.....	46	.....	94	75
Mannite	28	24	65	2	0	2	6	0	3
Raffinose	6	32	0	4	0	12	28	74	73

We find, as did Winslow, that dextrose is fermented by a larger number of the strains tested than any other sugar. A larger number of strains fermenting lactose was found by us than is reported by Winslow. In this respect our results show a closer agreement with those of Houston. We find also a higher percentage of streptococci in the cow dung capable of fermenting raffinose than is noted by Winslow. In this regard also our results are more nearly like those of the English observers. Houston reports 74 per cent of raffinose fermenting streptococci in cow dung, Winslow 28 per cent, and Fuller and Armstrong 75 per cent. The most important point of disagreement between our results and those of the other observers is in regard to the per cent of mannite fermenting organisms found in human feces. The per cent of mannite fermenting strains found by us in horse and cow dung corresponds closely to that noted by Winslow and Houston; but in the human feces we find them nearly three times as numerous as is found by either of the other observers. We recorded 65 per cent of mannite fermenting streptococci in human feces where Winslow and Houston find 28 per cent and 24 per cent respectively.

By correlating the results of the fermentation tests in each

separate carbohydrate several distinct types of streptococci can be quite clearly distinguished. We find for example that there is one group of organisms characterized by the fermentation of dextrose only, another characterized by the fermentation of dextrose and lactose, and a third by the fermentation of dextrose, lactose, and raffinose, etc. These results are expressed in the following table together with those published by Winslow in 1910.

TABLE 6.

SHOWING THE PERCENTAGE OF STRAINS FERMENTING DIFFERENT GROUPS OF SUGARS AS REPORTED BY WINSLOW, ANDREWES AND HORDER, AND FULLER AND ARMSTRONG.

CARBOHYDRATE FERMENTED	SPECIES	HUMAN		HORSE		COW	
	Andrewes and Horder's Types	Winslow	Fuller and Armstrong	Winslow	Fuller and Armstrong	Winslow	Fuller and Armstrong
None	.....	9	3	15	13	18	14
Dextrose only	Equinus	23	3	73	55	27	4
Lactose only	.....	2	0	0	1	5	1
Dextrose and Lactose	Mitis	31	30	5	18	21	5
Dextrose and Raffinose	.....	0	0	3	7	3	4
Lactose and Raffinose	.....	0	0	0	0	12	2
Dextrose, Lactose and Raffinose	Salivarius	5	0	0	4	9	64
Dextrose, Lactose and Mannite	Fecalis	23	65	0	1	2	2
All four	.....	0	0	1	0	3	3

For purposes of comparison we will for the moment consider the fermentative action of these cultures in dextrose, lactose, mannite, and raffinose only, since these carbohydrates were used by Winslow in his work. The more prevalent types are those isolated from horse dung fermenting dextrose only; two strains isolated from human feces, one of which ferments dextrose and lactose and the other which ferments dextrose, lactose, and mannite; and a group isolated from cow dung which ferments dextrose, lactose, and raffinose. From examination of Table 6 it will be seen that the numerical frequency with which these types were found to occur varies somewhat from that reported by Winslow.

Winslow's work indicates a considerably wider distribution of streptococci fermenting dextrose only than is indicated by our results. This observer found that 23 per cent of the human strains, 73 per cent of equine, and 27 per cent of bovine strains fermented dextrose only. This type would correspond to Andrewes and

Horder's *Str. equinus*. Our results show the presence of only 3 per cent of this type in human feces, 4 per cent in cow dung, and 55 per cent in horse dung. We have isolated this dextrose fermenting *Str. equinus* in considerable numbers in excreta of the horse only.

Dextrose and lactose fermenters, corresponding to Andrewes and Horder's *Str. mitis*, were found to be quite numerous in human feces, less so in horse dung, and in very small numbers in cow dung. Thirty per cent of the human strains fell in this group, 18 per cent of the equine, and 5 per cent of the bovine strains. Winslow's results correspond very closely with ours in this regard, 31 per cent of his cultures belonging to this type. He found this type to be less numerous in horse dung and in considerably larger numbers in cow dung than is indicated by our results. Winslow reports only 5 per cent of equine strains belonging to this group as against 18 per cent recorded by us and 21 per cent of bovine strains as against 5 per cent.

Streptococci fermenting dextrose, lactose, and raffinose, corresponding to Andrewes and Horder's *Str. salivarius*, were found abundantly in the excreta of cows but not in the feces of any of the other species examined. Sixty-four of the strains isolated from cow dung are of this type. No streptococci corresponding to this type were found in human feces and but 4 per cent of those obtained from the horse belongs to this group. Winslow, on the other hand, finds that 5 per cent of the human strains fermented dextrose, lactose, and raffinose; no organisms of this type were found in horse dung and only 9 per cent of the bovine strains fermented these sugars. Our results indicate the presence of a much larger number of streptococci of this type in cow dung than has been observed by Winslow.

Strains which ferment dextrose, lactose, and mannite were abundant in human feces. Sixty-five of the streptococci isolated from this source are included in this class which corresponds to Andrewes and Horder's type *Str. fecalis*. One per cent of the strains from the horse dung and 2 per cent from cow dung are of this type. We are in close agreement with Winslow as to the distribution of this group of streptococci in the excreta of the horse and cow, but find that it is far more abundant in human feces than is reported by this observer. Sixty-five per cent of the strains from the human

feces fermented dextrose, lactose, and mannite while Winslow includes in this group but 23 per cent of the cultures isolated from man.

The four types of streptococci described above are relatively more abundant in feces than any others. Several other types, however, are observed with less frequency.

One group found in the excreta of all the species examined failed to ferment any of the carbohydrates tested. A like group of non-fermenting organisms was also noted by Winslow. Strains which fermented lactose alone are not abundant. None were observed in human feces. One per cent of the strains from the horse and one per cent of those from the cow fermented saccharose only. Winslow reports 2 per cent of the human and 5 per cent of bovine strains which attack lactose only. None of these types were isolated from horse dung.

Organisms fermenting dextrose and raffinose were found in small numbers in the feces of both the horse and the cow by Winslow and ourselves.

Organisms fermenting lactose and raffinose were observed in cow dung but not in cultures from human and equine feces.

A few streptococci fermenting dextrose, lactose, mannite, and raffinose were found in cow dung but not in human and equine excreta. One per cent of the cultures isolated by Winslow from horse dung fermented dextrose, lactose, mannite, and raffinose. If we take into consideration the action of these organisms upon saccharose, very little change is found to be made in the main type centers recorded in the above correlations. By the introduction of saccharose into these calculations 15 groups appear. The type fermenting dextrose only (*Str. equinus*) is broken up into two groups, one fermenting dextrose only and the other fermenting dextrose and saccharose, the latter corresponding to the *Str. equinus* of Andrewes and Horder. The other important groups remain practically the same. The results obtained from the fermentation of inulin and salicin by fecal streptococci have not been included in this work for the reason that these tests were not introduced until after the experiments were well under way and therefore we have no records of the action of human strains upon these sugars.

SUMMARY.

From the results of these experiments it appears:

1. That streptococci producing a high acidity in dextrose media are in general characteristic of human excreta. These strains which produce between 3.5 per cent and 4.0 per cent acidity in this medium are relatively abundant in human excreta. Dextrose fermenting strains are less abundant in the excreta of the horse and cow, and produce an acidity considerably less than that of the human strains.

2. That streptococci fermenting mannite are present in human feces in large numbers but are almost entirely lacking in the excreta of the horse and the cow. Strains fermenting dextrose, lactose, and mannite (Andrewes and Horder's *Str. fecalis*) comprise 65 per cent of the cultures isolated from human stools, while only 1 per cent of the strains from the horse and 2 per cent of those from the cow are of this type.

3. That streptococci fermenting lactose are comparatively rare in horse dung.

4. That streptococci fermenting raffinose are abundant only in cow dung. Sixty-four per cent of the strains isolated from this source ferment dextrose, lactose, and raffinose (Andrewes and Horder's *Str. salivarius*), 2 per cent ferment lactose and raffinose, and 4 per cent, dextrose and raffinose. Streptococci of the salivarius type were not observed in human excreta, and comprised but 4 per cent of the strains isolated from horse dung.

CONCLUSIONS.

The bacterial flora of human feces is characterized in general by the presence of streptococci producing between 3.5 per cent and 4.0 per cent of acid in dextrose media. The prevailing type is *Str. fecalis* which ferments dextrose, lactose, and mannite. *Str. mitis* which ferments dextrose and lactose is also found in considerable numbers.

Horse dung contains few streptococci which ferment lactose. The predominating type is *Str. equinus* which ferments dextrose and saccharose.

The streptococci of cow dung are characterized by their power to ferment raffinose. The majority of strains isolated from this source are of the *Str. salivarius* type which ferments dextrose, lactose, and raffinose.

These results accord in general with those of Winslow and Palmer. They differ chiefly in the numerical frequency with which certain types occur in the different species examined. For example, Winslow reports that but 23 per cent of the strains isolated from human feces are of the *Str. fecalis* type, while our results indicate that a much larger proportion (65 per cent) of the strains from this material are of this type fermenting dextrose, lactose, and mannite. Winslow reports that 73 per cent of the equine streptococci belong to the *Str. equinus* type while but 55 per cent of the strains isolated by us from horse dung are of this type. Winslow reports but 9 per cent of *Str. salivarius* in cow dung while our tests indicate the presence of this type in 64 per cent of the strains isolated from this source.

Further experiments are now in progress with the view to testing the practical value of the fermentative activities of fecal streptococci in carbohydrate media for the differentiation of human and animal pollution in water supplies. In this work we are using the water samples received in the State Hygienic Laboratory for routine analysis.